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Equine endometrial cytology and bacteriology: Effectiveness for predicting live foaling rates

Davies-Morel, Mina Clare Gwynne; Lawlor, Olga Marion Francis; Nash, Deborah Mary

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Equine endometrial cytology and bacteriology: Effectiveness for predicting live foaling rates



M.C.G. Davies Morel, O. Lawlor, D.M. Nash*

Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Penglais, Aberystwyth, Ceredigion SY23 3AL, UK

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ABSTRACT

Endometritis is an important cause of sub-fertility in mares. The critical indicator of reproductive success and financial return for commercial studs is live foaling rate. Endometrial bacteriology and/or cytology are used to diagnose endometritis and thus identify mares at risk of early embryonic death. However, mares with endometritis may conceive but then abort in late gestation. The aims of this study were to establish, as part of a standard breeding examination (1) whether a threshold percentage of uterine polymorphonuclear neutrophils (PMNs) exists above which a significant reduction in live foaling rate is evident; (2) the relationship of a positive bacteriology result to live foaling rate, and (3) the relationship of a combination of positive cytology and bacteriology result to live foaling rate.

Guarded endometrial swabs ($n = 2660$) were collected from 1621 Thoroughbred mares on 17 commercial stud farms by five veterinarians during a single breeding season. All mares were included regardless of age, history or parity. Cytological and bacteriological analyses were performed on each swab and subsequent live foaling rates recorded. Data were analysed by comparing 0%, $\geq 1\%$, $\geq 2\%$, $\geq 5\%$ or $\geq 25\%$ PMNs of total cells counted, or categories of bacterial growth to live foaling rates, using Pearson's chi-squared test. A threshold value of $\geq 1\%$ PMNs, culture of a single bacterial isolate and a combination of both these parameters were associated with significantly reduced live foaling rates. Positive cytology alone, positive bacterial culture alone, or combined positive cytology and bacteriology were equally indicative of the likelihood of a mare producing a live foal.

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Introduction

In the United Kingdom in 2007 the aggregate value of public Thoroughbred sales was £302 million¹ (British Horseracing Authority, 2009) making the equine breeding industry a significant contributor to national and international economics. However, mares, particularly Thoroughbreds, exhibit poor reproductive efficiency (Sullivan et al., 1975; Morris and Allen, 2002). According to Thoroughbred Studbook Weatherbys, in 2011, of 7390 Thoroughbred mares bred in the UK, only 62.5% produced a live foal.²

Uterine inflammation (endometritis) is judged to be the most important gynaecological condition of horses (Traub-Dargatz et al., 1991; Troedsson, 1999; Card, 2005) and is the most common cause of embryonic loss before 35 days in normally cycling mares (LeBlanc, 2003). Endometritis occurs when foreign molecules, often spermatozoa and/or bacteria, are introduced into the reproductive tract, specifically at mating and/or due to general opportunistic bacterial contamination (Watson, 2000; Riddle

et al., 2007; LeBlanc, 2008). Opportunistic organisms typically include Gram positive (e.g. *Streptococcus zooepidemicus*; *S. zooepidemicus*) and Gram negative (e.g. *Escherichia coli*; *E. coli*) bacteria (Riddle et al., 2007; LeBlanc, 2008).

The inflammatory response to uterine challenge is characterised by an influx of polymorphonuclear neutrophils (PMNs), resulting in uterine luminal fluid and endometrial secretion of luteolytic prostaglandin (PG) $F_{2\alpha}$, which are incompatible with pregnancy (Watson et al., 1987; Pycock and Newcombe, 1996).

Uterine cytology and bacteriology are commonly employed in commercial practice as part of the pre-breeding examination in order to diagnose endometritis and its infectious or define non-infectious status and so indicate the likelihood of pregnancy success (Riddle et al., 2007; LeBlanc, 2010). However, there are differences in the guidelines for interpreting cytology (Card, 2005). Various cytological parameters are considered indicative of endometritis, for example: $\geq 10\%$ PMNs observed in a high power field (HPF) (Baranski et al., 2003); $>0.5\%$ PMNs of total cells counted (Ricketts and Mackintosh, 1987); 2–10% PMNs of total cells (Crickman and Pugh, 1986).

It has been reported that the use of bacteriology alone or in combination with cytology does not necessarily improve success

* Corresponding author. Tel.: +44 1970 622575.

E-mail address: dmn@aber.ac.uk (D.M. Nash).¹ £1 = approx. \$1.55, €1.19 at 4 May 2013.² See: www.weatherbys.co.uk (accessed 4 May 2013).

in diagnosing endometritis, although it does allow the identification of any causal agent (Riddle et al., 2007; LeBlanc, 2010). Several investigators have related pre-defined cytological or bacteriological characteristics to pregnancy rate although the parameters used have varied. Riddle et al. (2007) determined that the 28 day pregnancy rate was 7–36% when a micro-organism was cultured and/or a positive cytology (>2 PMNs per field) was observed, which was significantly lower than in mares negative for both examinations (60%).

Baranski et al. (2003) examined mares during foal heat and observed a pregnancy rate (date of pregnancy diagnosis not specified) of 18.2% in mares with positive cytology (≥ 10 PMN per HPF) and bacteriology, and in mares where either cytology or bacteriology was positive pregnancy rates were 18–23%, compared to 54% in negative animals. Most recently no correlation of positive cytology (using a variety of assessment criteria) or bacteriology was reported to be associated with 70 day pregnancy rate (Nielsen et al., 2012).

No work has been reported to examine whether there is a critical threshold of PMNs detected in relation to pregnancy outcome. Furthermore, early pregnancy rates used as an indicator of success do not reflect the most critical measure of reproductive efficiency and financial return, namely the production of a live foal (Carrick and O'Meara, 2010). Mares with endometritis may conceive but experience bacterial placentitis and abortion as late as 120 days of gestation (Asbury, 1983; LeBlanc, 2003).

The aims of this study were to determine whether, as part of a standard breeding examination, a threshold percentage of uterine PMNs could be determined above which a significant reduction in live foaling rate is evident. We also examined the relationship of positive bacteriology to live foaling rate and the relationship of a combination of positive cytology and bacteriology results to live foaling rate.

Materials and methods

Animals and sample collection

A total of 2660 guarded uterine endometrial swabs were collected from 1621 Thoroughbred mares on 17 commercial stud farms by five veterinarians from the same practice in the Upper Hunter Valley region of New South Wales in Australia (latitude 32°5'S, longitude 150°52'E). The swabs were collected as part of routine management and thus represented a random population of mares encountered in practice, and were all analysed at the same in-house practice laboratory. Maiden, foaling, barren and problem mares were included and all had endometrial swabs collected for cytological and bacteriological analysis prior to mating.

Mares were bred on subsequent oestrus periods by natural cover, normally within 12 h of ovulation. Generally, animals were not bred on foal heat, with the exception of some when the end of the breeding season was approaching. Mares found to have a positive bacteriology were treated with intra-uterine antibiotics, Intervet Neomycin–Penicillin, every 24 h from the time of diagnosis until 48 h post cover. Several mares had multiple swabs collected throughout the season which were included in the study. Immediately after collection the swabs were placed in a charcoal free AMIES transport medium and transferred within a few hours to the laboratory.

Cytology

Each swab was rolled onto a frosted microscope slide and left to air dry. A commercial Romanowsky-type stain kit (Fronine, Thermo Fisher Scientific) was used and slides were prepared according to manufacturer instructions. Specimens were evaluated under a light microscope at $\times 40$ and under oil at $\times 100$ magnification. Seventy-seven (2.5%) of samples were hypocellular samples and were discarded. A minimum of 100 cells in ten fields were counted and percentage PMNs of the total cells observed was determined.

Bacteriology

Swabs were plated on horse blood agar (HBA; Oxoid Australia) and incubated for 48 h at 36.7 °C. The plates were checked at 24 and 48 h for bacterial growth. Bacterial growth was classified as: no growth; mixed flora (two or more organisms); or

single isolate (one bacterial isolate only). Where bacterial growth was detected Gram positive or Gram negative organisms were identified using Gram stain smears (Thermo Fisher).

Live foaling rate

Live foaling rate was determined from the Australian and New Zealand stud books.

Data analysis

All swabs taken from mares within the season along with the first and the last swab of the season prior to mating were analysed. All swabs were included in order to ascertain whether a swab taken at any time of the season was indicative of problems in addition to the more obvious first and last swab.

When assessed for cytology (investigation 1) samples were assigned to groups according to % PMNs of total cells counted: 0 (all swabs $n = 2487$, first swab $n = 1514$, last swab $n = 1529$); $\geq 1\%$ (all swabs $n = 173$, first swab $n = 106$, last swab $n = 91$); $\geq 2\%$ (all swabs $n = 125$, first swab $n = 73$, last swab $n = 64$); $\geq 5\%$ (all swabs $n = 79$, first swab $n = 50$, last swab $n = 29$), and $\geq 25\%$ (all swabs $n = 35$, first swab $n = 23$, last swabs $n = 18$). Foaling rates for each classification were compared to 0% PMN. Data were analysed for all swabs, the first swab of the season and for the last swab collected from the mare prior to the final mating of the season. The lower threshold values (0%, $\geq 1\%$, $\geq 2\%$, $\geq 5\%$ PMNs) were chosen to encompass typical parameters reported by existing literature (Ball et al., 1988; Overbeck et al., 2011). The upper value ($\geq 25\%$ PMNs) was chosen to insure the widest range of possible thresholds was considered. An individual sample may have appeared in more than one PMN group, for example, a sample that yielded 10% PMNs appeared in both $\geq 2\%$ and $\geq 5\%$ PMN groups but not in the $\geq 25\%$ PMN group.

When assessed for bacteriology (investigation 2a) foaling rates were compared for each classification (no growth, mixed flora, single isolate). Data were analysed for all swabs, first and last swab collected from the mare prior to the final mating of the season.

Investigation 2b considered the positive bacteriology samples observed in all swabs, first swab and the last mare swab of the season and were divided into Gram positive and Gram negative groups and compared to foaling rate.

Investigation 3 combined cytology and bacteriology where foaling rates were compared for samples that exhibited positive cytology only ($\geq 1\%$ PMNs), a single isolate only (positive bacteriology), or a paired single isolate culture result with positive cytology. This was done for all swabs, first and final swabs of the season.

Pearson's chi-square (χ^2) analysis was used throughout to determine if significant differences existed between foaling rates. Significance was assumed where $P < 0.05$. Once parameters were determined for cytology (investigation 1), bacteriology (investigation 2a) and combined cytology and bacteriology (investigation 3), the sensitivity, specificity, negative predictive value and positive predictive value were calculated for each, using live foaling rate as the best standard. The sensitivity was the proportion of mares that had a positive diagnosis of all mares that did not produce a live foal (true positive). The specificity was the proportion of mares that had a negative diagnosis of all the mares that produced a live foal (true negative). The negative predictive value was the proportion of mares that were truly negative (negative diagnosis and produced a live foal) of all negatively diagnosed animals. The positive predicted value was the proportion of mares that were truly positive (positive diagnosis and did not produce a live foal) of all positively diagnosed animals.

Results

Investigation 1

Of all the samples that were collected ($n = 2660$), 2487 (93.5%) had no PMNs present and were associated with a foaling rate of 66.9%. In comparison to these PMN negative samples, those that exhibited $\geq 1\%$ PMN had a significantly reduced foaling rate (group $\geq 1\%$ PMNs, $P < 0.05$; $\geq 2\%$ PMNs, $P < 0.01$; $\geq 5\%$ PMNs, $P < 0.05$) with the exception of $\geq 25\%$ PMNs (Fig. 1).

For the first swab of the season ($n = 1621$), 1514 (93.4%) mares had no PMNs present, related to a foaling rate of 74%. In comparison to these PMN negative samples, swabs that exhibited $\geq 2\%$ PMN had a significantly reduced foaling rate ($P < 0.05$) and those with $\geq 5\%$ had a tendency for a reduced foaling rate ($P < 0.07$). No effect was observed for the $\geq 1\%$ and $\geq 25\%$ PMNs (Fig. 2).

For the final swab of the season ($n = 1621$), 1529 (94.3%) mares had no PMNs present, related to a foaling rate of 74%. In comparison to these PMN negative samples, swabs that exhibited $\geq 1\%$ and

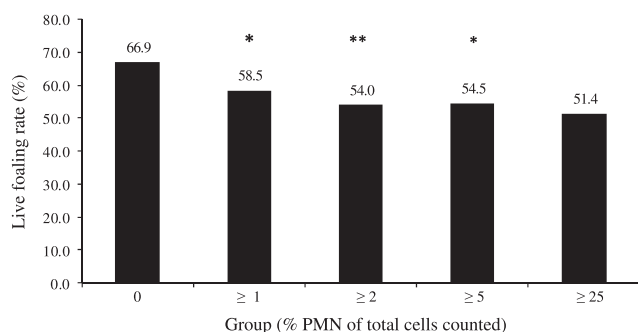


Fig. 1. Relationship between % PMNs in all swabs and live foaling rates. Live foaling rate for mares with samples of % PMNs was significantly different from samples with ≥ 1 PMNs ($*P < 0.05$), ≥ 2 PMNs ($**P < 0.01$), ≥ 5 PMNs ($*P < 0.05$).

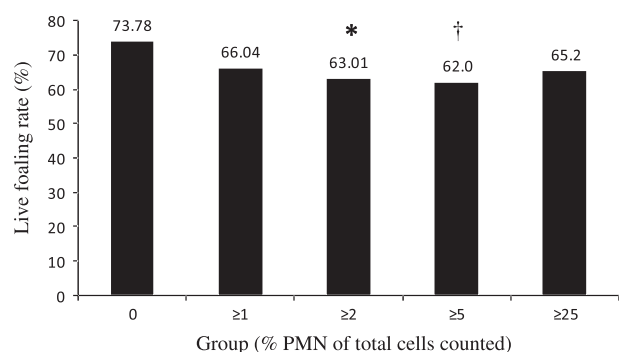


Fig. 2. Relationship between % PMN in first swab of the season and live foaling rates. Live foaling rate of mares with samples of 0% PMNs was significantly different than samples with ≥ 2 PMNs ($*P < 0.05$) and a tendency to be different for samples with ≥ 5 PMNs ($†P < 0.07$).

2% PMN had a significantly reduced foaling rate ($P < 0.05$) but no effect was observed for the ≥ 5 and 25% PMNs (Fig. 3).

Investigation 2a

Of all the samples that were collected ($n = 2660$), 2475 (93.1%) mares demonstrated no bacterial organism on culture and had a foaling rate of 67.3%. Those with mixed flora ($n = 84$) resulted in a 59.3% foaling rate, which was not significantly different to mares with no growth. Samples where a single isolate was observed ($n = 101$) exhibited a 49.5% foaling rate which was significantly ($P < 0.001$) lower than mares with no bacterial growth (Fig. 4).

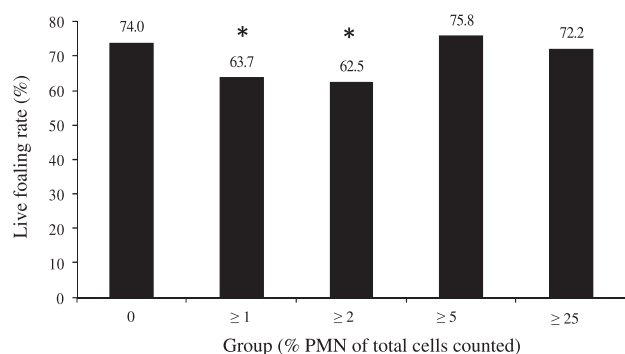


Fig. 3. Relationship between % PMN in last swab of the season and live foaling rates. Live foaling rate of mares with samples of 0% PMNs was significantly different than samples with ≥ 1 PMNs and ≥ 2 PMNs ($*P < 0.05$).

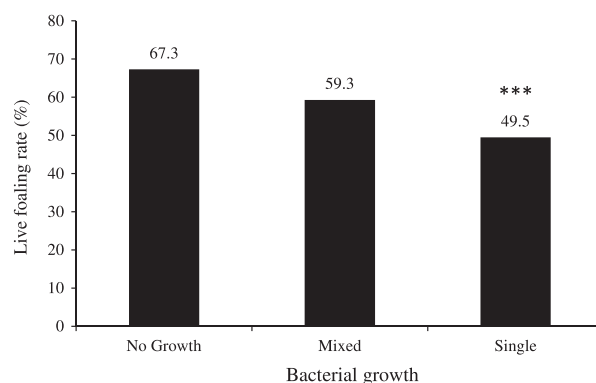


Fig. 4. Relationship between foaling rates and bacterial organisms isolated in all swabs. Live foaling rate was significantly lower ($***P < 0.001$) from mares with a single isolate compared to mares with no growth.

For the first swabs of the season ($n = 1621$), 1524 (94.0%) mares demonstrated no bacterial organism and had a foaling rate of 74.2%. Mares with mixed flora ($n = 46$) and those with a single isolate ($n = 51$) exhibited a 71.7% and 51% foaling rate respectively. Foaling rate for single isolate was significantly lower than both mixed ($P < 0.05$) and no growth ($P < 0.001$) (Fig. 5).

For the final swabs of the season ($n = 1621$), 1533 (94.6%) mares demonstrated no bacterial organism and had a foaling rate of 73.6%. Mares with mixed flora ($n = 46$) and those with a single isolate ($n = 42$) exhibited a 67.4% and 70% foaling rate respectively. Neither was significantly different to mares with no growth (Fig. 6).

Investigation 2b

When all swabs, first swabs and final swabs were considered there was a consistent significant difference in foaling rate between Gram positive and Gram negative cultures (all swabs Gram positive 63%, Gram negative 31%, $P < 0.01$; first swabs Gram positive 66%, Gram negative 24%, $P < 0.01$; final swabs Gram positive 84%, Gram negative 47%, $P < 0.05$).

Investigation 3

For all samples that were collected ($n = 2660$), samples that had a single isolate only ($n = 64$) had a foaling rate of 43.8%. Samples where a positive cytology (≥ 1 PMNs) only was observed

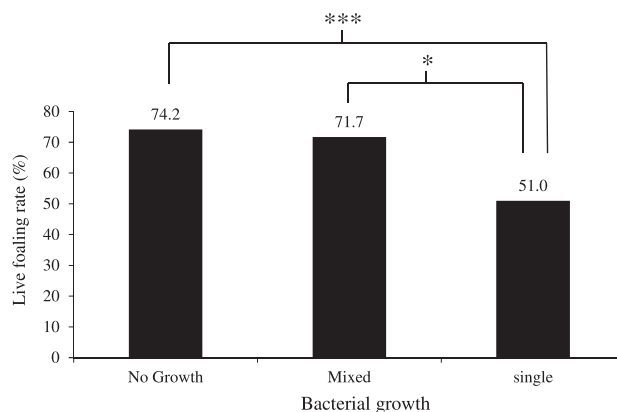


Fig. 5. Relationship between foaling rates and bacterial organisms isolated in first swabs of the season. Live foaling rate was significantly lower for mares with a single isolate compared to both mixed ($*P < 0.05$) and no growth ($***P < 0.001$).

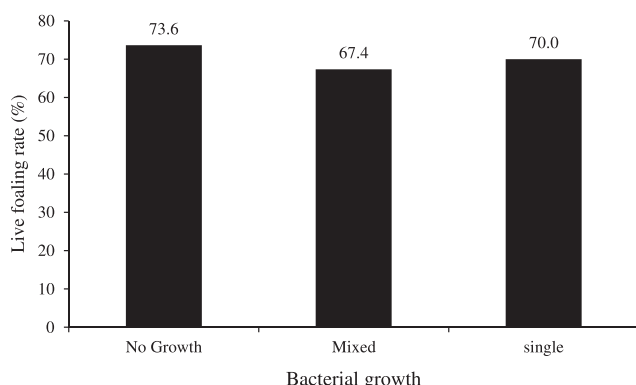


Fig. 6. Relationship between foaling rates and bacterial organisms isolated in final swabs of the season. No significant differences were evident.

($n = 121$) exhibited a 58.7% foaling rate. Samples where a single isolate was coupled with a positive cytology result ($\geq 1\%$ PMN's; $n = 36$) exhibited a 58.3% foaling rate. These findings did not significantly differ (Fig. 7).

For the first swabs of the season ($n = 1621$), samples that had a single isolate only ($n = 31$) related to a foaling rate of 45.2%. Samples where a positive cytology ($\geq 1\%$ PMNs) only ($n = 78$) exhibited a 65.4% foaling rate. Samples where a single isolate was coupled with a positive cytology result ($\geq 1\%$ PMNs; $n = 20$) exhibited a 60.0% foaling rate. These findings were not significantly different from each other (Fig. 8).

For the final swabs of the season ($n = 1621$), samples that had a single isolate only ($n = 22$) related to a foaling rate of 69.6%. Samples where a positive cytology ($\geq 1\%$ PMNs) only ($n = 65$) exhibited a 61.5% foaling rate. Samples where a single isolate was coupled with a positive cytology result ($\geq 1\%$ PMNs; $n = 17$) exhibited a 70.6% foaling rate. These findings were not significantly different from each other (Fig. 9).

Sensitivity, specificity and predictive values

Sensitivity for all parameters determined for cytology, bacteriology or a combination of both, for all, first or last swabs ranged between 0.01 and 0.08. Specificity for each parameter ranged between 0.94 and 0.99. The negative and positive predicted value for each parameter ranged between 0.67 and 0.75 and 0.29 and 0.51, respectively (Table 1).

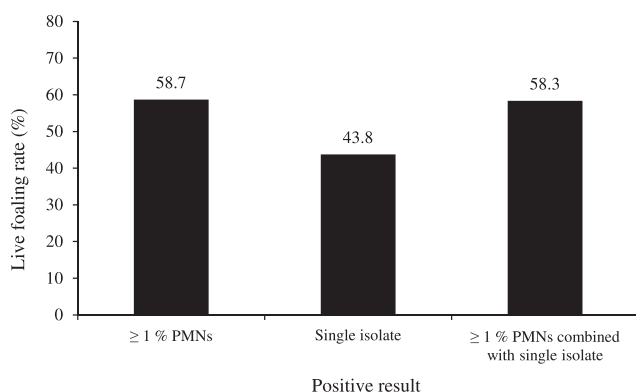


Fig. 7. Relationship between foaling rates and mares demonstrating $\geq 1\%$ PMNs only, single isolate only, or a combination of $\geq 1\%$ PMNs and single isolate for all swabs. No significant differences were evident.

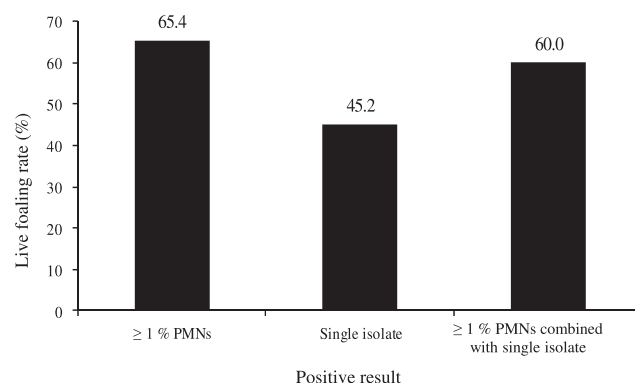


Fig. 8. Relationship between foaling rates and mares demonstrating $\geq 1\%$ PMNs only, single isolate only, or a combination of $\geq 1\%$ PMNs and single isolate for first swabs of the season. No significant differences were evident.

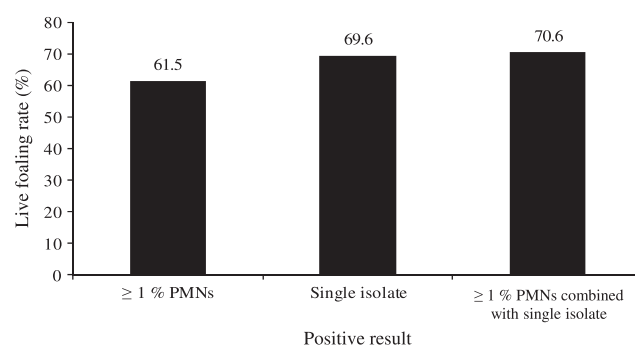


Fig. 9. Relationship between foaling rates and mares demonstrating $\geq 1\%$ PMNs only, single isolate only, or a combination of $\geq 1\%$ PMNs and single isolate for final swabs of the season. No significant differences were evident.

Discussion

This investigation used a large data set of 2660 swabs collected during a single breeding season from over 1600 mares in stud practice under routine veterinary guidance. Of this large data set the vast majority of samples were negative for cytology (94.3%) or grew no bacteria in culture (93.1%). This agrees with previous work, for example Riddle et al. (2007), who reported 90.1% of samples demonstrated negative cytology. Despite this, the current study presents a robust investigation of particular relevance to commercial veterinary practice. The ultimate purpose for any stud is the production of live foals. Contrary to previous work which examined pregnancy rates (Baranski et al., 2003; Riddle et al., 2007; Nielsen et al., 2012) our study used live foaling rate as an indication of reproductive success, which is particularly relevant to stud managers.

A threshold of $\geq 1\%$ PMNs of total cells resulted in significantly lower foaling rates compared to mares that exhibited no PMNs whatsoever ($P < 0.05$) in all and last swabs. Significance was reached at $\geq 2\%$ PMN in first swabs of the season. Therefore, a threshold value of $\geq 1\%$ PMN above which live foaling rates are reduced is justified, particularly for all or final swabs. This $\geq 1\%$ PMN threshold that we identified was greater than that suggested by Ricketts and Mackintosh (1987; $\geq 0.5\%$ PMNs), yet lower than that recommended by others where ≥ 3 – 10% PMNs were considered diagnostic of inflammation (Crickman and Pugh, 1986; Ball et al., 1988; Overbeck et al., 2011). Nielsen et al. (2012) found that the presence of PMNs was the only significant factor impacting day 70 pregnancy rates when detected histologically, via biopsy, rather than cytology. However, there is a limit to the feasibility of using endometrial biopsies in commercial practice. Riddle et al. (2007)

Table 1
Sensitivity, specificity, negative and positive predictive value of cytology (>1% PMNs), bacteriology (single isolate) or a combination of both to diagnose uterine inflammation in the mare, calculated using live foaling rate as the best standard. Data were collected from all swabs collected from each mare during the breeding season or the first or last swab of the season.

	Cytology (>1% PMN)			Bacteriology (single isolate)			Combined cytology and bacteriology (>1% PMNs and single isolate)		
	All swabs	First swabs	Last swabs	All swabs	First swabs	Last swabs	All swabs	First swabs	Last swabs
Sensitivity	0.08	0.08	0.08	0.06	0.06	0.03	0.02	0.02	0.01
Specificity	0.94	0.94	0.95	0.97	0.98	0.98	0.99	0.99	0.99
Negative predictive value	0.67	0.74	0.74	0.67	0.74	0.74	0.68	0.75	0.74
Positive predictive value	0.42	0.34	0.36	0.51	0.49	0.30	0.42	0.40	0.29

stated that 28 day pregnancy rates were dependent on the severity of inflammation (diagnosed where >2 PMNs present per HPF) and our data substantiate this in relation to foaling rate.

For all swabs, beyond the $\geq 1\%$ PMN threshold, a further reduction in foaling rate occurred (54.0%, 54.5% and 51.4% for $\geq 2\%$, $\geq 5\%$ and $\geq 25\%$ PMNs respectively versus 58.5% for the threshold value). The present study collected samples before breeding throughout a season from non-selected, commercial mares, including maiden, normal and abnormal mares, and from a range of ages and parities, with individuals treated by antimicrobials as and when directed by the veterinarian; thus the assessments of inflammation and foaling rates in the study represent the typical population encountered in stud practice.

Despite using an extensive population of animals, a particularly anomalous result was evident with regard to the final swabs where $\geq 5\%$ and $\geq 25\%$ PMN groups were related to relatively high foaling rates. This may be explained, at least in part, by the very low number of cytology samples within these groups. Additionally the owners of these few mares were determined to achieve a foal regardless of effort or cost as the end of the season approached and would have included breeding and swabbing on foal heat (not typical of the other mares in this study). Mares swabbed during foal heat exhibit an increased inflammatory response, indicated by uterine cytology (Belz and Glatzel, 1995; Reilas et al., 2000; Baranski et al., 2003) that may or may not be linked to reduced pregnancy rates. Despite this, it can be concluded that $\geq 1\%$ PMNs may be considered a threshold above which a significant decrease in foaling rate is evident.

The culture of a single isolate, but not mixed growths, was associated with significantly lower foaling rates than samples where no bacteria were detected, when considering all and first swabs (both $P < 0.001$). Riddle et al. (2007) also demonstrated that culture of a bacterial pathogen was associated with decreased pregnancy rates after 28 days. Data from the current study further imply that single isolates are most detrimental to foaling rate, which agrees with the long-standing belief that pure cultures are associated with a more acute endometritis (Wingfield Digby and Ricketts, 1982; LeBlanc, 2003). Overbeck et al. (2011) and Nielsen et al. (2012) suggested that the presence of three organisms or more (i.e. mixed cultures) were the result of general contamination; hence the mixed growths found in the present study may represent commensal species with a relatively low level of pathogenicity and inflammation.

In total, single isolates accounted for only 4.1% of all samples collected, lower than that of Overbeck et al. (2011), where 9.1% *S. zooepidemicus* and 10.1% *E. coli* monocultures were observed, and far fewer than 40.0% pure cultures reported by Wingfield Digby and Ricketts (1982). However, Overbeck et al. (2011) used a total of only 55 samples from barren mares and Wingfield Digby and Ricketts (1982) used unguarded swabs which were likely to have resulted in sample contamination, compared to the present study which utilised guarded swabs on a large and broad population of mares. In particular, Gram negative bacteria were associated with low foaling rates, which may be due to the formation of biofilms

during chronic infections providing protection against host inflammatory responses and antibiotics (LeBlanc et al., 2007).

Thus, a single isolate, irrespective of the cytology result, was associated with decreased foaling rates for all and first swabs. Although this observation agrees with Riddle et al. (2007), albeit in relation to 28 day pregnancy rate, it contrasts with the traditional view that a true positive bacterial culture result only occurs when paired with inflammatory cytology (Wingfield Digby and Ricketts, 1982; Card, 2005; Causey, 2006).

There was no significant effect on foaling rate of either single isolate or mixed growth observed for the final swab of the season. This may be explained by the high proportion of mares that were approaching the end of the breeding season in this group, as discussed for cytology.

As a similar percentage of mares positive for cytology ($\geq 1\%$ PMN), or a single bacterial isolate or a combination of both, failed to produce a foal it can be concluded that all three parameters were equally indicative. This challenges the traditional view that positive bacteriology in the absence of paired positive cytology is likely to be a false result (Crickman and Pugh, 1986; LeBlanc et al., 2007). The occurrence of misleading bacteriological findings has been associated with the use of unguarded swabs (Card, 2005). We addressed this in the present study by using guarded swabs. Furthermore, if the detection of bacteria included false positives, a greater foaling rate would have been expected compared to cytology alone or a combination of the two; this was not evident. Likewise, the accuracy of cytology alone has also been questioned (Nielsen, 2005; Overbeck et al., 2011). Nonetheless, despite the rather low number of mares in each group, the similarities in foaling rate between cytology alone, bacteriology alone and a combination of the two, does not suggest any major inaccuracies in any one technique used, at least in the current study.

Some mares had several swabs taken throughout the season and so our analysis included first, all and last swabs. Collecting more than one swab in a season is common practice and our investigation justifies the cost of collecting and analysing numerous swabs. Despite treatment with antibiotics following every 'dirty swab', the cytology threshold and bacteriology results obtained from any swab taken in the season, as well as first and last swab, are indicative of poor live foal rates.

Considering the parameters determined here (>1% PMNs, single isolate or a combination of both), the true negatives (specificity) for these were notably high in that all were greater than 0.94. In contrast the true positives (sensitivity) were especially low with all below 0.08. Existing studies have also calculated sensitivity, specificity and predictive values of similar methods for diagnosing uterine inflammation (Nielsen, 2005; LeBlanc et al., 2007). For example, Nielsen (2005) determined sensitivity for cytology and bacteriology as 0.77 and 0.34 and specificity as 1.0 and 1.0, respectively. However, the best standard used to calculate these values used by Nielsen (2005) and LeBlanc et al. (2007) was biopsy diagnosis of inflammation to evaluate diagnostic techniques compared

to each other, whereas we examined the value of diagnostic parameters compared to a clinical outcome, namely, a live foal.

The clinical meaning of our data is that an individual with a negative diagnosis is highly likely to produce offspring. This is substantiated by the negative predictive values that ranged between 0.67 and 0.75; these values are favourable with observed live foaling rate and that expected from Thoroughbreds in practice (62.5–82.7%; Morris and Allen, 2002) and as indicated by Weatherbys.³ However, a mare with a positive diagnosis may still produce a live foal. Indeed, using all swabs as an example, foaling rates were 58.5% (positive cytology), 49.5% (positive bacteriology) and 58.3% (positive for both). Nevertheless, despite having a chance of foaling, these rates are significantly lower than those of clean mares (>66.9%). Biologically and clinically a foaling rate of <60% on a breeding farm is considered poor performance.

Conclusions

A threshold level of $\geq 1\%$ PMNs was determined, above which a significant reduction in foaling rate was evident. This, or the presence of a positive, single, bacterial isolate detected from any swab taken within the season was associated with a significant decrease in foaling rate. Additionally, this study indicates bacteriology alone, cytology alone or a combination of both are equally indicative of foaling rate and may therefore be similarly advocated for use in commercial veterinary practice to advise on the likelihood of reproductive success for a particular mare.

Conflict of interest statement

None of the authors have any financial or personal relationships with other people which may have inappropriately influenced the content of this work.

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References

- Asbury, A.C., 1983. Bacterial endometritis. In: Robinson, N.E. (Ed.), *Current Therapy in Equine Medicine*. W.B. Saunders, Philadelphia, pp. 410–414.
- Ball, B.A., Shin, S., Patten, V., Lein, D., Woods, D.L., 1988. Use of a low-volume uterine flush for microbiologic and cytologic examination of the mare's endometrium. *Theriogenology* 29, 1269–1283.
- Baranski, W., Janowski, T., Ras, A., Podhalicz-Dzielgielewska, M., Strzerek, R., 2003. Relationship between bacteriological and cytological examination of the mare's uterus during foal heat and fertility rate. *Bulletin of the Veterinary Institute of Pulawy* 47, 427–433.
- Belz, J., Glatzel, P., 1995. Fertility in mares after a disturbed as well as undisturbed puerperium. Significance of histological and cytological examinations of the uterus. *Tierarztl Prax* 23, 267–272.
- British Horseracing Authority, 2009. Economic Impact of British Racing. British Horseracing Authority, City.
- Card, C., 2005. Post-breeding inflammation and endometrial cytology in mares. *Theriogenology* 64, 580–588.
- Carrick, J.B., O'Meara, D., 2010. Use of mare breeding records to predict fertility. In: Australian College of Veterinary Scientist 2010 Annual Conference, Brisbane.
- Causey, R., 2006. Making sense of equine uterine infections: The many faces of physical clearance. *The Veterinary Journal* 172, 405–421.
- Crickman, J.A., Pugh, D.G., 1986. Equine endometrial cytology: A review of techniques and interpretations. *Veterinary Medicine* 81, 650–656.
- LeBlanc, M., 2003. The mare. In: Knottenbelt, D., LeBlanc, M., Lopate, C., Pascoe, R. (Eds.), *Equine Stud Farm Medicine and Surgery*. Elsevier Science Limited, Edinburgh, pp. 113–211.
- LeBlanc, M.M., 2008. When to refer an infertile mare to a theriogenologist. *Theriogenology* 70, 421–429.
- LeBlanc, M.M., 2010. Advances in the diagnosis and treatment of chronic infectious and post-mating-induced endometritis in the mare. *Reproduction in Domestic Animals* 4, 21–27.
- LeBlanc, M.M., Magsig, J., Stromberg, A.J., 2007. Use of a low-volume uterine flush for diagnosing endometritis in chronically infertile mares. *Theriogenology* 68, 403–412.
- Morris, L.H.A., Allen, W.R., 2002. Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. *Equine Veterinary Journal* 34, 51–60.
- Nielsen, J., 2005. Endometritis in the mare: A diagnostic study comparing cultures from swab and biopsy. *Theriogenology* 64, 510–518.
- Nielsen, J., Nielsen, F., Petersen, M.R., 2012. Diagnosis of equine endometritis – Microbiology, cytology and histology of endometrial biopsies and the correlation to fertility. *Pferdeheilkunde* 28, 8–13.
- Overbeck, W., Witte, T.S., Heuwieser, W., 2011. Comparison of three diagnostic methods to identify subclinical endometritis in mares. *Theriogenology* 75, 1311–1318.
- Pycok, J.F., Newcombe, J., 1996. The relationship between intraluminal fluid, endometritis, and pregnancy rate in the mare. *Equine Practice* 18, 19–22.
- Reilas, T., Huhtinen, M., Oksanen, M., Katila, T., 2000. Relationship between embryo recovery rate and uterine leavage fluid composition in postpartum mares. *Reproduction and Nutrition Development* 40, 383–391.
- Ricketts, S.W., Mackintosh, M.E., 1987. Role of anaerobic bacteria in equine endometritis. *Journal of Reproduction and Fertility Suppl.* 35, 343–351.
- Riddle, W., LeBlanc, M., Stromberg, A., 2007. Relationships between uterine culture, cytology and pregnancy rates in Thoroughbred practice. *Theriogenology* 68, 395–402.
- Sullivan, J.J., Turner, P.C., Self, L.C., Gutteridge, H.B., Bartell, D.E., 1975. Survey of reproductive efficiency in the Quarter-horse and Thoroughbred. *Journal of Reproduction and Fertility Suppl.* 23, 315–318.
- Traub-Dargatz, J.L., Salman, M.D., Voss, J.L., 1991. Medical problems of adult horses, as ranked by equine practitioners. *Journal of the American Veterinary Medical Association* 198, 1745–1747.
- Troedsson, M.H., 1999. Uterine clearance and resistance to persistent endometritis in the mare. *Theriogenology* 52, 461–471.
- Watson, E.D., 2000. Post-breeding endometritis in the mare. *Animal Reproduction Science* 60–61, 221–232.
- Watson, E.D., Stokes, C.R., David, J.S., Bourne, F.J., Ricketts, S.W., 1987. Concentrations of uterine luminal prostaglandins in mares with acute and persistent endometritis. *Equine Veterinary Journal* 19, 31–37.
- Wingfield Digby, N.J., Ricketts, S.W., 1982. Results of concurrent bacteriological and cytological examinations of the endometrium of mares in routine stud farm practice 1978–1981. *Journal of Reproduction and Fertility Suppl.* 32, 181–185.

³ See: www.weatherbys.co.uk.